The Effect of GSM Mobile Phone Electromagnetic Field on Femur Fracture Healing in a Rat Model

Cep Telefonlarının Yaydığı Elektromanyetik Alanın (EMA) Sıçan Modelinde Femur Kırık İyileşmesi Üzerine Etkisi

Orijinal Araştırma JCAM Original Research

Cep telefonunun Kırık İyileşmesine Etkisi / The Effect of Mobile Phone on Fracture Healing

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Özet

Amaç: Son zamanlarda cep telefonlarının elektromanyetik alanın biyolojik etkileri ve insan sağlığına etkiler araştırmacıların çok dikkatini çekmiştir. Bu çalışmada amaç 900 MHz ile çalışan cep telefonlarının sıçan modellerinde femur kırık iyileşmesi üzerine etkisini araştırmaktır. Gereç ve Yöntem: Altmış adet Sprague-Dawley sıçanda anestezi altında sağ femur kapalı kırığı oluşturuldu. Hemen arkasından kapalı redüksiyon sonrası 21 G enjektör iğnesi ile tespit yapıldı. Bunlardan otuz adet sıçan EM grubuna alınarak 7 gün boyunca 1 saat/gün süresince 900 MHz radyasyon (2 W peak output power ve 1.04 mW/cm2 power density) uygulandı. Diğer otuz adet sıçan ise kontrol grubuna alınarak doğal iyileşme sürecine bırakıldı. Kırık iyileşmesi ikinci ,4. ve 6. haftalarda radyolojik (Lane and Sandhu sınıflandırması), histolojik (Huo skalası) ve biyomekanik (3-point bending) olarak değerlendirildi. Bulgular: Histopatolojik ve radyolojik incelemede EM ve kontrol grubu arasında kırık iyileşmesi arasında fark izlenmedi. Biyomekanik incelemede 2. haftada EM grubunda kontrol grubundan istatistiksel olarak daha anlamlı bir hızlı iyileşme izlendi ($p<0.05$). Ancak 4. ve 6. haftada biyomekanik fark izlenmedi. Sonuç: 900 MHz frekanslı cep telefonlarının yaydığı elektromanyetik alanın (EMA) sıçan kırık modellerinde kırık iyileşmesi üzerine belirgin bir etkisi izlenmedi.

Anahtar Kelimeler

Kırık İyileşmesi; Elektromanyetik Alan; Cep Telefonu; Sıçan

Abstract

Aim: Biological effects of electromagnetic field (EMF) and their consequences on human health have been the subject of much interest and research in recent years. The aim of this study was to investigate the effects of 900 MHz EMF on femur fracture healing in a rat model. Material and Method: After sixty male Sprague-Dawley rats were exposed to a closed right femur fracture under anesthesia, the reduction and fixation were done with a 21 g needle. Then, 900 MHz radiation (2 W peak output power and 1.04 mW/cm2 power density) was applied to EM group for one hour/day for seven days. The healing was assessed using radiological (Lane and Sandhu classification), histological (Huo scale for callus evaluation), and biomechanical (3-point bending) measures at 2nd, 4th and 6th weeks after fracture. Results: Fracture healing, as assessed radiologically and histopathologically, in Group EM and control animals was similar at 2nd, 4th and 6th weeks. Fracture healing, as assessed biomechanically, was significantly better in Group EM compared to controls in those sacrificed at 2nd week post-procedure ($p<0.05$). Biomechanical strength was not different between the groups at 4th and 6th weeks. Discussion: 900 MHz EMF from a mobile phone in this rat femur fracture model resulted in no significant difference in healing from controls not exposed to EM radiation.

Keywords

Fracture Healing; Electromagnetic Field; Mobile Phone; Rat

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Introduction

The use of GSM mobile phones has been increased rapidly over the last 20 years; recent estimates state that over one billion GSM mobile phones are now in use around the world. Biological effects of electromagnetic field (EMF) and their consequences on human health have been the subject of much interest and research in recent years.

GSM mobile phones operate using mainly 900 and 1800 MHz frequency bands, frequencies at which organic molecules (including water) are excited, causing thermal and non-thermal effects [1]. Cellular responses to ionizing, ultraviolet, and electromagnetic radiation are classified as reversible or irreversible, and can result in structural or functional changes [2]. To date, microwave radiation from GSM mobile phones and base stations has not been found to be dangerous to humans [3-5]. However, microwave emissions from mobile phones have been reported in some studies to have beneficial effects on human tissues [6,7].

Sert et al. reported no significant changes in mean serum calcium, magnesium, lithium, and creatinine levels after rats were exposed to 50 MHz electromagnetic radiation [8]. When postmenopausal women were treated with free-transition magnetic waves (12 minutes a day for 30 days, then 24 minutes a day for 150 days), an increase in osteocalcin, procollagen, estrone, and estradiol levels; a decrease in serum total and partially ionized calcium levels; and an improvement in DXA values were observed [9].

EM radiation at a frequency of 15 Hz in pulse form has a therapeutic effect on osteoporosis [10]. Chang and Chang suggested that pulse EM radiation has a regulatory effect on trabecular bone structure by decreasing the loss of trabecular bone tissue in an experimental model in bilateral oopherectomised rats [10]. Zhang et al. reported an increase in mean bone mineral density in osteoporotic rats after EM radiation treatments at a frequency of 8.0 Hz and a magnetic power of 0.4 T for 30 minutes a day for 30 days [11]. All of these studies were done with EM radiation at low frequencies, but mobile phones operate using higher frequencies (900 and 1800 MHz).

Acceleration of fracture healing has been attempted with both pharmaceuticals and physical interventions. EM radiation also has been found to have effects on cellular systems, and may have beneficial effects on fracture healing [1,3,6]. An animal model for studies of the effects of EM on fracture repair has yet to be performed. The goal of our study was to investigate the effect of one hour/day for seven days of 900 MHz EM from a mobile phone in a rat model of femur fracture healing at 2, 4, and 6 weeks after the fracture.

Material and Method

This experimental study was approved in our university Institutional Animal Care and Use Committee, and surgical procedures were done under the guidance of the Division of Laboratory Animal Resources at our university. This experimental study is performed between 06.01.2009 and 20.08.2009.

Animals

Sixty young male Sprague-Dawley rats (weighing 220-290 gr) were used in the study. The animals were fed a standard rat diet (free access to standard rat chow and tap water), but were deprived of food 12 hours before the femur fracture procedure.

Study Design

Rats were assigned randomly to 2-, 4- and 6-week groups. Each of these groups of 20 rats was further divided equally into an EM radiation-exposed group and a control group. The right femur of all rats was fractured (as described below), then, as described below, the Group EM rats were exposed to radiation. After two, four, and six weeks had elapsed from the time of surgery, 10 Group EM and 10 Group control (Group C) rats were sacrificed, as described below. In summary, 10 rats were in each of the following treatment groups:

1st Group EM, sacrificed at 2nd week of the fracture,
2nd Group control, sacrificed at 2nd week of the fracture,
3rd Group EM, sacrificed at 4th week of the fracture,
4th Group control, sacrificed at 4th week of the fracture,
5th Group EM, sacrificed at 6th week of the fracture and
6th Group control, sacrificed at 6th week of the fracture.

Surgical technique for fracturing the femur

Each rat in Group EM and Group C was prepared with ketamine HCl (75 mg/kg) and xylazine HCl (5 mg/kg). The soft tissues overlying the right knee joint were gently and sharply dissected. With the leg in extension, the patellar ligament was lateralized and under flexion, an access hole into the intramedullary canal was created with a 21-gauge, 0.8 mm diameter hypodermic needle (Hayat Medical Products, Istanbul, Turkey). The needle was inserted into the intramedullary canal, then was pulled back without exiting the intercondylar notch. The femur was then broken mechanically by the Bonnarens and Einhorn method [12]. After closed reduction of the fractured femur, the needle was inserted into the femur again in a retrograde manner and left in place as an intramedullary pin. It was then cut to the appropriate length and the cut off piece of needle was set aside. The knee incision was closed in layers with 4-0 Vicryl® sutures and the rat was returned to its cage. Plain X-rays were taken within an hour to examine the fracture pattern and adequacy of fixation.

Exposure to electromagnetic energy

A device with six antennas generated a modulated (217 Hz repetition frequency) 900 MHz pulse electromagnetic wave (2 W peak output power and 1.04 mW/cm2 power density). The power density and specific absorption rate (SAR) measurements were performed at the Electromagnetic Compatibility (EMC) Laboratory of Department of Electronics and Communication Engineering (Suleyman Demirel University, Isparta, Turkey). Exposure to EM radiation began the day after surgery and occurred with the rat in a plastic tube cage (length 12 cm, diameter 5 cm) with a dipole antenna near the head of the rats. The tube was ventilated from head to tail to decrease the stress of the rat while in the tube. Group EM rats were exposed to the EM radiation for one hour per day for seven consecutive days.

Necropsy

Rats were euthanized at 2, 4 and 6 weeks post-surgery, using 200 mg/kg of ketamine HCl (if needed, an additional dose was given). The operated and contra-lateral non-operated femurs were retrieved, radiographs of the femurs were taken and then the intramedullary needle was delicately removed. The rat femurs were then fixed in 10% neutral buffered formaline for mechanical testing and histopathological examination.

Radiography

Radiographs were taken of all samples with a Hitachi X-ray machine (Model U-6CE-55-TB), at settings of 25 kilovolts, 2 milliamps for 9 s on shelf number 8. By placing the specimens on the same shelf number in the exposure chamber, a constant distance between the X-ray source and specimen was obtained. This procedure was performed within 30 minutes of necropsy. All radiographs were evaluated by one orthopedic surgeon and one radiologist. Results were classified according to the Lane and Sandhu system which scores fracture healing as 0 (no callus), 1 (callus formation present but no evidence of bone union), 2 (initiation of bone union present), 3 (bone union present, fracture line no longer visible), or 4 (complete bony union)[13].

Biomechanical Testing

Specimens (both healing fractured femurs and non-fractured healthy femurs) were thawed to room temperature before mechanical testing, and were kept moist in formaline solution throughout the experiment. This procedure was performed within four hours of necropsy. Evaluations of the right femurs were made using the three-point mechanical stress test reported by Nakamura et al [14]. Specimens were placed on a metal holding device with supports 15 mm apart, and the device was connected to an actuator (TA-XT2i Texture Analyzer, Stable Micro Systems Ltd., Godalming, UK). A load (measured in Newtons, N) was applied to stress the bone until fracture midway between the supports on the anterior surface at a speed of 10 mm/min. Just after biomechanical testing, the bones were again placed in formalin solution.

Histopathologic procedures

Following the biomechanical testing, the same femur specimens were prepared for histopathology. Samples were stored in 10% neutral buffered formaline for four days and then decalcified for 15 days in 10% formic acid, embedded in paraffin, and sectioned by microtome longitudinally. Then, they were stained with hematoxyline-eosin and examined under light microscope by 100x magnification. The pathologist who examined the histopathological sections was blinded to the purposes of the study and group allocation of the animal. The fracture callus in each specimen was quantified according to the Huo scale [15], giving a possible histopathological score of 1 to 10 as follows: 1 (fibrous tissue only), 2 (predominantly fibrous tissue with some cartilage), 3 (equal amounts of fibrous tissue and cartilage), 4 (all cartilage), 5 (predominantly cartilage with some trabecular bone), 6 (equal amounts of cartilage and trabecular bone), 7 (predominantly trabecular bone with minimal cartilage), 8 (entirely trabecular bone), 9 (mostly trabecular bone with some mature lamellar bone), and 10 (mature lamellar bone). Osteoblast activity was not recorded routinely nor analyzed statistically because it was not a part of a standard measurement scale, such as the Huo scale [15].

Statistical analyses

Statistical analyses were performed with SPSS for Windows® 11 software. Findings at 2, 4, and 6 weeks postoperatively in Group EM and Group C rats were compared with Mann-Whitney U testing. Differences were considered significant when $p<0.05$.

Results

Procedures were completed without protocol violation in any study animals. No infections occurred in any of the animals.

At 2 weeks post-surgery, radiological evidence of fracture healing was seen in both EM and control groups (Lane and Sandhu score of 1-2, Figure 1-A, Table 1). At week 4, the fracture line was less distinct (Lane and Sandhu score of 2-3, Figure 1-B, Table 1) and at week 6, complete union was observed (Lane and Sandhu score of 3-4, Figure 1-C, Table 1).

At 2 weeks post-surgery, histopathological evidence of fracture healing (predominantly cartilage, Huo grade 5) was seen in both Group EM and C rats (Figure 2-A, Table 1). Osteoblasts were seen more often in Group EM than in Group C specimens. At week 4, bone tissue became prominent and cartilage maturation was observed (Huo grade 7, Figure 2-B, Table 1) and at week 6, mature bone tissue was observed (Huo grade 8, Figure 2-C, Table 1). The mean Huo grade of Group C rats was slightly better than Group EM that of rats at 2, 4, and 6 weeks, but these differences were not statistically significant.

Bone strength was significantly greater in Group EM rats at 2 weeks ($p<0.05$), but at 4 weeks, bone strength in the two groups was similar ($p=0.44$). At 6 weeks, bone strength in Group EM rats was greater, but not by a significant amount ($p=0.36$). As can be seen in Figure 3, bone strength of Group EM rats at 2 weeks post-fracture was almost the same as bone strength of Group C rats at 4 weeks.

Discussion

In this rat femur fracture model, daily exposure to one hour of 900 MHz EM radiation had beneficial effects early in the course of healing (at two weeks); but no difference was observed between controls and irradiated rats at 4 and 6 weeks after the fracture.

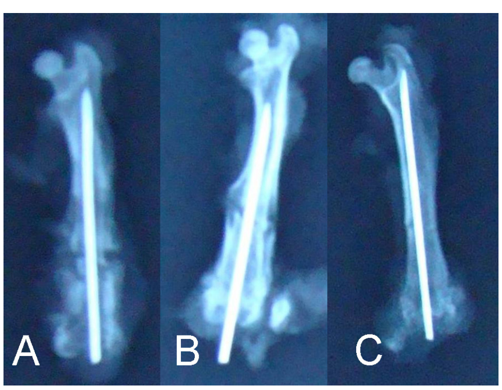


Figure 1. Radiographic appearance of rat femur fracture healing at 2 weeks post-fracture (Lane and Sandhu score 1-2) (A), at 4 weeks post-fracture (Lane and Sandhu score 2-3) (B), at 6 weeks post-fracture (Lane and Sandhu score 3-4) (C).

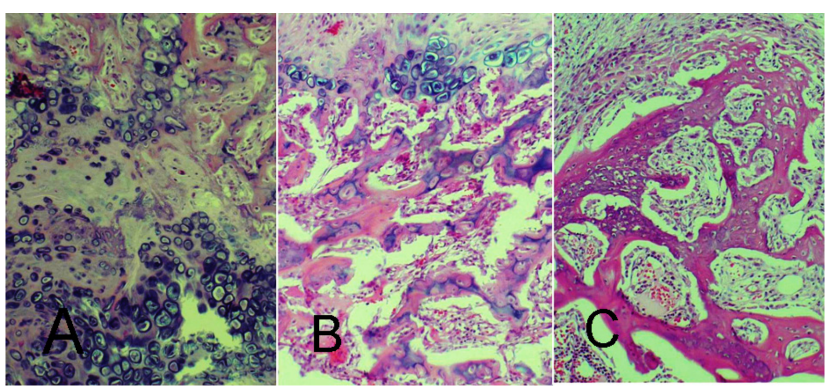
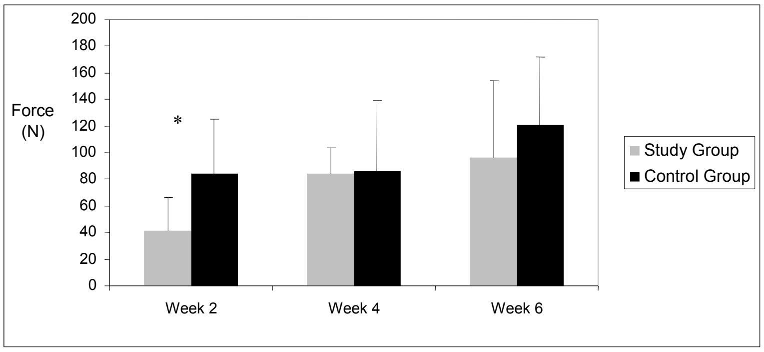


Figure 2. Histopathological appearance of rat femur fracture healing at 2 weeks post-fracture (Huo grade 5) (A), at 4 weeks post-fracture (Huo grade 7), 2-C: at 6 weeks post-fracture (Huo grade 8) (B).

Figure 3. Force applied until fracture (N=Newtons) of rat femurs [controls and those exposed to 900 MHz electromagnetic radiation (EM Group) for one hour per day for seven days] at 2, 4, and 6 weeks post-fracture.



Mobile telephones emitting 900 MHz EM radiation are commonly used in many countries; they have a power output of about 2W, which produces a minimal amount of local heating that has not yet been associated with any gross deleterious effects. EM radiation has been shown to cause changes in living tissues such as altering the permeability of the blood-brain barrier, encephalogram and blood pressure, mostly through 'non-thermal' pathways [16]. Lower frequency EM fields appear to be more bioactive than higher frequency fields of the same rest characteristics [17]. Extremely low frequency (ELF) EM fields, of the order of several V/m, as occurring with both GSM (Groupe Spécial Mobile) and DCS (digital cellular system) transmissions, are able to disrupt cell function by irregular gating of electro sensitive ion channels on the plasma membranes of cells [18].

Panagopoulos et al. reported that EM radiation from mobile telephones causes a decrease in oviposition due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells [19]. Although cell death due to mobile phone EM radiation was reported in the study by Panagopoulos et al., we found no deleterious effect on fracture healing; on the contrary, two weeks after the fracture, the EM radiation-exposed animals were healing slightly faster than the control group. Bassett et al found that EM radiation in proper dosage and duration accelerated fracture healing (osteotomy of the radius) in a rat model [20]. They declared that this positive effect of EM radiation on different cell types may vary according to frequency, amplitude, and wave pattern.

Koyu et al. published a study showing that EM radiation lowered thyroid stimulating hormone (TSH) and thyroid hormone levels [21]. These effects may be due to heating of the tissues, effects which are similar to nonspecific stress responses induced by EM radiation in rats. Increased production of local growth factors, such as bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-b), growth differentiation factors (GDF), IL-1, IL-6, TNF-a , PDGF, and activins early on may have resulted in accelerated fracture healing. As exposure continues for weeks, adverse effects may cancel out any beneficial effect, to result in an insignificant difference from controls. Testing these hypotheses would require measurement of hormone levels in future studies of fracture healing and EM radiation.

In a study of 61 randomly selected patients who had previously failed to respond to pre-operative conservative treatment for discogenic low back pain, Marks et al. showed that pulsed electromagnetic field (PEMF) stimulation enhanced bony bridging after lumbar spinal fusions and afforded a better clinical outcome [22]. They used PEMF stimulation at least 4 hours a day beginning 2 days postoperatively and continuing for 2-4 months post-operatively. Similarly, Borsalino published PEMF stimulation was found to be beneficial in patients with degenerative hip arthritis treated with femoral intertrochanteric osteotomy [23]. We doubt that the low EM energy emitted by 900 MHz mobile phones would have such positive effects in a clinical setting.

In other experimental studies, EM radiation of 0.4 T for 30 minutes a day for 30 days at 15 MHz and 8 MHz was found to have a beneficial effect on osteoporosis [11]. A regulatory effect of EM radiation (2 hours per day for 9 days) on trabecular bone structure in bilateral oopherectomised rats was found by Chang and Chang [10]. Tsai et al reported stimulation of human mesenchymal stem cells (hMSCs) by rectangular pulse PEMF radiation (7.5 Hz for 2 hours per day for 14 days) [24]. His study results indicate that extremely low frequency PEMF stimulation may play a modulating role in human mesenchymal stem cell osteogenesis. Smith et al reported the mechanism of PEMF to be its significant arteriolar vasodilatation effects on tissue [25].

We observed better fracture healing in EM radiation-exposed animals 2 weeks after fracture, but not after 4 or 6 weeks. Our results were perhaps not as good as they could have been, had we exposed the rats to longer periods (more than one hour) of EM radiation per day, or for more days (longer than seven days), or at a lower frequency (less than 900 MHz). We used 900 Mhz because that is a standard transmission frequency of mobile telephones, but it is much higher than the frequencies of EM radiation used in experimental healing research, for example, in the studies by Chang, Zhang and Tsai [10, 11, 39]. Low frequency electromagnetic radiation may have more therapeutic potential in fracture healing than high frequency EM radiation [11, 22, 23]. Although human fractures were not studied, the results of the present study suggest the need to investigate the effects of EM radiation from mobile phones on bone healing in humans. Although EM radiation was related to faster bone healing after 2 weeks as measured in our biomechanical test, radiological and histopathological results do not support the accelerating effect of EM radiation on fracture healing.

Short duration 900 MHz EM radiation for 7 days may promote fracture healing as measured by increased biomechanical strength and osteoblastic activity in the early phases of bone healing. As proposed by other researchers, even greater beneficial effects on fracture healing may be obtained from EM radiation of lower frequencies. Further studies in small and larger animals should be performed before testing such procedures on human subjects.

Exposure to electromagnetic radiation (900 MHz) as emitted from commonly used mobile telephones for one hour per day for one week does not have any adverse effects on fracture healing in this rat femur fracture model, as measured via radiologic, histopathologic and biomechanical parameters. It may have some mild beneficial effects on bone healing in the first two weeks after fracture.

Disclosure: All authors declare that they have neither conflict of interest nor financial relationships with any product or procedure mentioned in the manuscript.

Conflict of interest: All authors certifies that they have no conflict of interest related to the manuscript and that they have no financial relationships or benefits from a commercial entity related to this work or with any product used in the research project. All authors certify also that nobody has any commercial associations that might pose a conflict of interest in connection with the submitted article.

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